STAIN TECHNOLOGY

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PROGRESS IN THE STANDARDIZATION OF STAINS

STANDARDIZATION OF STAINING SOLUTIONS

In the last issue of this journal, attention was called to the fact that the forthcoming edition of the National Formulary is to contain formulae of staining solutions which have been standardized in coöperation with the Stain Commission. Still another step is shortly to be taken in the direction of standardizing staining solutions which will be of interest to those users of stains who find it most convenient to buy their solutions already prepared.

Two recent notes[1] in the Journal of the American Medical Association have contained preliminary announcements of this plan. The plan is not yet quite ready to put into operation, altho it is hoped that it will be before the end of the current year. When it does go into effect, it will work out something as follows:

A selection is being made at present of a number of reliable concerns thruout the country which prepare and sell staining solutions. When this list is completed, it is hoped to have some such concern to coöperate with us in all of the large centers thruout the country. The names of concerns for this list are being selected upon the recommendation of members of the Stain Commission.

When the list is complete, it is planned to ask each concern to submit to us samples of certain staining solutions; at the start we will begin with only one or two of those (notably Wright stain) which are most frequently purchased in solution form. After testing such a solution, it is the plan to give the concern submitting it information as to whether it meets our tests and if not as to how it may be improved so as to do so. After any one of these concerns has thus demonstrated its ability to make up a satisfactory solution of any particular stain, we will furnish them labels to be used on this product stating essentially as follows:

> This certifies that this solution was made with dyes certified by the Commission on Standardization of Biological

[1]Conn, H. J. 1938. The work of the Commission on Standardization of Biological Stains. J. Am. Med. Assn. **111**, 1239–40.

————. 1939. Certification of staining solutions. *Id.*, 112, 1281.

Stains and according to the directions of the National Formulary. It was prepared on.....

These labels will be sufficiently different in style from the regular certification labels so that they will not be confused with them. They will be sold to the dispensaries of the staining solutions at a small price per label.

As a check on the situation, it is planned to institute some kind of inspection system so that occasional samples of these solutions are purchased by Commission members on the open market and sent to the chairman's laboratory for testing. It is hoped in this way to make certain that the quality of such solutions is maintained.

It is also intended to publish the names, from time to time, of the concerns which are coöperating in this way, together with lists of staining solutions which have been put on this basis. This will enable users of such solutions to learn where they can purchase them.

Finances of the Stain Commission

A recent report in Science[2] has called attention to the fact that the Stain Commission is no longer to receive any financial assistance from the Chemical Foundation. This situation has been brought about because of the large decrease in the Foundation's income which has taken place upon the expiration of the patents that it has owned. It is in no sense to be regarded as due to loss of interest of the Chemical Foundation in the work of the Stain Commission. This is well evidenced by the following quotation from a letter just received from Mr. W. W. Buffum of the Foundation: "The Chemical Foundation is justly proud of the progress of the Commission on Standardization of Biological Stains. The total contributions by the Chemical Foundation from 1921 to date to your work have been $100,999.98, and we feel that your accomplishments during this period have more than justified this expenditure."

From the standpoint of continuing the work of the Stain Commission, it is unfortunate that the Chemical Foundation's appropriations must now be discontinued, but it is not so serious as it would have been a few years ago. For several years the size of these appropriations has been gradually reduced and the balance has been made up from the Commission's earned income. Had the Foundation been able to continue the partial support of this work, at a progressively reduced rate, for some five or six years longer, the probabilities are that by then the Commission would be entirely self-supporting. It

[2]Conn, H. J. *et al.*, 1939. Financial status of the Biological Stain Commission. Sci., 89, 367–8.

is accordingly possible at the present time to assure any other source from which funds are requested that they will be needed only to take care of a temporary shortage. Even without appropriations from any outside source, the deficit will not be great; a matter of $2,000 a year should balance the budget completely.

While it is much to be regretted that the support of the Chemical Foundation has to cease before the work of the Commission is entirely self-supporting, our feeling toward the Foundation is one of gratitude that it has been able to continue the support for so long—nearly twenty years, as a matter of fact. To appreciate the importance of this support, one should realize that when the standardization of stains was first undertaken, no one had any thoughts that it could be made self-supporting. The manufacture of stains was a very small branch of the dye industry which in itself was a "war baby", just getting on its feet. To have saddled the business of biological stain production with the expenses involved in standardization would have killed it at the start. Altho the National Research Council was very much interested in the project, they had no funds of their own available, and it was only because of the vision of the late Mr. F. P. Garvan and the generosity of the Chemical Foundation that the necessary financial support was forthcoming. It is not the least exaggeration to say that without this support, the work could never have been started. Accordingly, users of stains who feel that this standardization has been of value, owe a very considerable measure of gratitude to the Chemical Foundation for its assistance.

As we have every reason to believe that the work is going to prove self-supporting eventually, our chief hope at present is that some organization with a little money available will come to the rescue and tide us over the present emergency.—H. J. Conn.

THE PRESENT MEMBERS OF THE COMMISSION ON STANDARDIZATION OF BIOLOGICAL STAINS

It has been several years since the last publication[1] of the list of members of the Stain Commission. In the meantime, there have been numerous changes, both in personnel and in addresses of some of the older members.

It has been even longer since any statement has appeared in these pages as to the way the Commission is organized; and as there are today many readers of STAIN TECHNOLOGY who did not see the early issues as they appeared, it seems well to repeat some of the information as to the make-up of this organization. The Commission was organized in 1922 by a Committee of the National Research Council. Its original membership consisted of about fifty collaborators who had been assisting this committee during the preceding year in its work on the standardization of biological stains. According to the constitution of the Stain Commission: "Other persons shall be eligible to membership who establish to the satisfaction of the Executive Committee that they are interested in the work of the organization, and are ready, willing and able to assist in the work of the organization, if and at such times as assistance in his or her special field may be requested of such member by the Executive Committee. Any such person may be admitted to membership upon his application to membership receiving the approval of the members of the Executive Committee. Continuance of membership shall be evidenced by the members signing and returning to the Chairman of the Executive Committee semi-annually a declaration in substantially the following form: 'I desire to renew and continue my membership on the Commission on Standardization of Biological Stains.' "

This latter provision of the constitution has been observed by sending out a card to each member twice a year bearing essentially the above statement; failure to return two of these cards in succession has been interpreted as equivalent to resignation.

The total number of members has been kept between 50 and 75 by adding new individuals occasionally to replace those who have dropped out. These new members have been added by invitation only.

The following are active members at the present time:

EXECUTIVE COMMITTEE

H. J. Conn, Agricultural Experiment Station, Geneva, N. Y.
E. V. Cowdry, School of Medicine, Washington Univ., St. Louis, Mo.

[1] STAIN TECHNOLOGY, 9, 73. 1934.

Louis Gershenfeld, Phil. College of Pharm. & Science, Philadelphia, Pa.
H. E. Jordan, Univ. of Virginia, University, Va.
S. I. Kornhauser, School of Medicine, Univ. of Louisville, Louisville, Ky.
F. B. Mallory, Boston City Hospital, Boston, Mass.
J. T. Scanlan, Bureau of Chemistry & Soils, U. S. Dept. of Agriculture, Washington, D. C.
W. D. Stovall, Univ. of Wisconsin, Madison, Wis.

Members at Large

C. E. Allen, Biology Bldg., Univ. of Wisconsin, Madison, Wis.
Ezra Allen, 450 E. 64th St., New York, N. Y.
Elizabeth Bachelis, 105 Elliot Place, Bronx, N. Y.
H. W. Beams, Dept. of Zoology, State Univ. of Iowa, Iowa City, Ia.
Wm. M. Bright, Municipal College, Louisville, Ky.
Victor Burke, Washington State College, Pullman, Wash.
E. Eleanor Carothers, Dept. of Zoology, State Univ. of Iowa, Iowa City, Ia.
C. J. Chamberlain, Dept. Botany, Univ. of Chicago, Chicago, Ill.
Clyde Chandler, N. Y. Botanical Garden, New York, N. Y.
G. H. Chapman, 604 Fifth Ave., New York, N. Y.
E. C. Cole, Williams College, Williamstown, Mass.
Francis W. Constable, St. Joseph's Hospital, Providence, R. I.
H. A. Davenport, 303 E. Chicago Ave., Chicago, Ill.
A. B. Dawson, Biological Laboratories, Harvard University, Cambridge, Mass.
J. A. deTomasi, 220 Plant Science Bldg., Cornell University, Ithaca, N. Y.
C. R. Fellers, Mass. State College, Amherst, Mass.
N. C. Foot, New York Hospital, 525 East 68th St., New York, N. Y.
R. D. Fox, Dept. of Bact., University of Akron, Akron, O.
Elizabeth F. Genung, 42 West St., Northampton, Mass.
M. F. Guyer, Dept. of Zoology, Univ. of Wisconsin, Madison, Wis.
F. E. Hale, Mt. Prospect Laboratories, 355 Park Place, Brooklyn, N. Y.
Robert T. Hance, 1 Broadmoor Ave., Pittsburgh, Pa.
H. T. Herrick, Bureau of Chemistry & Soils, U. S. Dept. of Agric., Washington, D. C.
D. C. Hetherington, Duke Hospital, Durham, N. C.
E. G. Hood, Science Service, Dairy Research, Dept. of Agric., Ottawa, Canada.
Alfred E. A. Hudson, Waynesboro Hospital, Waynesboro, Va.
S. H. Hutner, 158 Knox St., Albany, N. Y.
D. A. Irwin, Banting Institute, 100 College St., Toronto, Canada.
M. W. Jennison, Biology Dept., Mass. Inst. Techn., Cambridge, Mass.
J. E. Kindred, Box 1341, University, Va.
Max Levine, Bact. Dept., Iowa State College, Ames, Ia.
Leon S. Lippincott, Box 231, Vicksburg, Miss.
H. Macy, University Farm, St. Paul, Minn.

W. L. Mallmann, Dept. of Bact., Mich. State College, East Lansing, Mich.
M. S. Marshall, Dept. of Bact., Univ. of Cal. Medical School, San Francisco, Cal.
C. E. McClung, Univ. of Pennsylvania, Philadelphia, Pa.
Roy L. Mobley, Box 1021, Baton Rouge, La.
J. T. Myers, Bendiner & Schlesinger Lab., 3rd Ave. & 10th St., New York, N. Y.
T. S. Painter, Univ. of Texas, Austin, Tex.
Alexander Petrunkevitch, Yale University, New Haven, Conn.
L. F. Randolph, Plant Science Bldg., Cornell Univ., Ithaca, N. Y.
P. W. Rohrbaugh, 549 Ocean View, Whittier, Cal.
Sara A. Scudder, City Hospital, Welfare Island, N. Y.
L. W. Sharp, Plant Science Bldg., Cornell University, Ithaca, N. Y.
Waldo Shumway, Univ. of Illinois, Urbana, Ill.
Karl A. Stiles, Coe College, Cedar Rapids, Ia.
F. W. Tanner, 362 Chemistry Bldg., Univ. of Illinois, Urbana, Ill.
Wm. R. Taylor, Dept. of Botany, Univ. of Michigan, Ann Arbor, Mich.
Alexander V. Tolstoouhov, 305 East 63rd St., New York, N. Y.
S. T. Walton, City Health Dept., Charlotte, N. C.
H. B. Ward, Univ. of Illinois, Urbana, Ill.
H. C. Waterman, 3218 Stephenson Place, N. W., Washington, D. C.

APPLICATION OF FINE GRAIN PROCESSING AND CONDENSER ILLUMINATION[1] ENLARGING TO PHOTOMICROGRAPHY

FRANK P. MCWHORTER, *Plant Pathologist, Oregon Experiment Station, Corvallis, Ore.*

ABSTRACT.—**Photomicrographs involving great resolution are usually made by means of long initial projection. This involves the use of heavy, often cumbersome, apparatus designed to eliminate vibration. This paper evaluates the possibility of using an intermediate projection distance equal to or greater than 160 mm., which is considered the minimum efficient distance, and recording the initial negative image in very fine grain. Then by critical enlarging a positive image is obtained which closely approaches the resolution obtainable by the finest long projection equipment. The initial short projection permits elimination of vibration difficulties attending long exposures so that critical printing at magnifications above 2000 × will give consistently good results, provided fine grain technic is mastered by the operator.**

Introduction: It is generally conceded that the limit of usable magnification for direct vision with compensating eyepieces is the numerical aperture of the objective multiplied by 1,000.[2] This goal is seldom attained satisfactorily even by experienced microscopists skilled in critical illumination methods because the human elements, eye strain, vision variations, and eye defects, restrict the final achievement. This article is intended to show how usable magnifications beyond the 1000 × aperture limit are obtainable with average eyepiece magnifications by combining recently developed fine grain processing with condenser enlarging. It is not asserted that such photographic magnification will increase resolution beyond the $\frac{\lambda}{2\,NA}$ law, but it is claimed that with a given set of lenses, these methods will (1) record details which cannot be seen with average vision and (2) enable any observer to notice details which he might not otherwise observe.

It is not claimed that the methods and ideas involved are new, nor that no other worker has ever combined fine grained processing with critical enlarging. Since the possibilities of the combination are not

[1]Published as Technical Paper No. 281 with the approval of the Director of the Oregon Agricultural Experimental Station. Contribution of the Department of Botany, and the Bureau of Plant Industry, U. S. D. A., coöperating.

[2]Belling, John. 1930. The use of the microscope. McGraw-Hill Pub. Co.

generally recognized, an analysis of the factors governing the results seems advisable.

Meaning of condenser illumination enlarging: The usual enlarger, including expensive "auto focus" models, is constructed with ground glass diffusion areas between the film holder and the light. Uniform illumination and partial diffusion for artistic effects are thus obtained. The light source is usually a frosted globe. The light scatters from the frosted surface in every possible angle, only a portion of it passing thru the film. In turn, the light transmitted to the lens is still diffusing. The lens must pick the rays moving in the right direction or turn those moving in the wrong direction into a cone which eventually reaches the enlarging paper. Most of the original light is wasted. If a properly adjusted condenser replaces the ground glass and an unfrosted concentrated filament lamp replaces the usual light source, we have the principles of critical illumination applied to photographic enlarging. The difference in performance is comparable to using a microscope with, versus without, a condenser; the optical principles are the same. The condenser in either case bends the light rays into a cone comparable to the numerical aperture of the lens system and eliminates unwanted diffused rays. A truly critical illumination enlarger is one in which the condenser system can be focused for the different positions which the projection lens assumes as different magnifications are obtained. For most purposes, however, one having a fixed condenser is very efficient. An excellent description of the optics involved was presented by H. C. Benedict in Camera Craft for June 1936.[3]

If a good negative portraying black lines close together is projected thru an ordinary enlarger, the lines will diffuse noticeably as the magnification increases beyond 2 ×, and will diffuse greatly beyond 3 ×. The better kinds of "Minicam" enlargers equipped with six element lenses but without condensers will project lines to 5 × with good effect. With critical illumination and microtessars, lines will project to 30 or more times—the emulsion permitting. In photomicrography in general, and certainly from the viewpoint of this article, we are dealing with line and dot projection; then even the best pictorial-purpose enlargers fail. Conversely, the more critical the enlarger, the more difficult it becomes to avoid graininess in the projected image.

The negative for enlarging: Photographs at high magnifications are usually obtained with special projection eyepieces and long bellows extensions necessitating elaborate mechanical devices to dampen vibration. The use of such equipment, altho time-consuming, is

[3]Benedict, H. C. 1936. A positive method for alternating the apparent exposure scale of a paper through the use of a condenser enlarger. Camera Craft, **23**, 275–284.

advisable for extreme resolution and magnification because objectives of high NA are cut with a theoretical projection point located at infinity. The image, however, when projected to a distance which permits very high initial magnification, tends to soften unless ideal illumination obtains. For this reason an image representing high initial magnification, unless the subject involves extreme contrast, requires a process film and a contrast developer for photographic recording. This concept is the basis for recommending the combination of the Wratten M plate and the developer D 19. (See footnote 2.)

The finest photomicrographs of minute difficult subjects are made with long initial image projection combined with tonal development as opposed to contrast development with D 19. This also permits secondary enlargement as described here. Dr. Max Poser's magnificent pictures of diatoms printed as glass transparencies at magnifications of 10,000 and more involve just such technic. Such work can be done only with exceptional equipment and by exceptional workers. The writer believes that anyone familiar with critical illumination principles can make good high magnification images more easily if relatively short initial projection distances are used in conjunction with fine grain processing. For routine work, a "ride the microscope" type of camera is advised. This type of camera accommodates plates up to the 9 × 12 centimeter size. If the microscope is lighted as in critical illumination and an eyepiece is used which is suitable for either looking thru or projection, then the good illumination which obtains for direct vision is also fine for projection, and very sharp images are present at 160 mm. from the microscope. Vibration difficulties, moreover, are largely eliminated.[4] The 160 mm. projection of the image represents an intermediate distance permitting the projected field to cover a 3¼ × 4¼ plate. It is far more usable than the "postage stamp size" initial images obtained by inverting a "candid" camera a short distance above a microscope. The making of a negative is a matter of moments—not hours. If the image is picked up at 160 mm. as with a Makam camera, the maintenance of contrast is less difficult and developers permitting gradation and tone rendering may be used; these developers may be chosen to eliminate graininess. The procedures herein recommended are based on the fact that the resolving power of a photographic emulsion is inversely proportionate to its graininess.

Tests of different developers: In order to obtain high magnification from relatively short initial projection, the negative must have fine grain and normal contrast. Unfortunately, the degree of gamma

[4] McWhorter, F. P. 1927. A simple and inexpensive method of making photomicrographs. The Camera, Dec. 1927, 381–385.

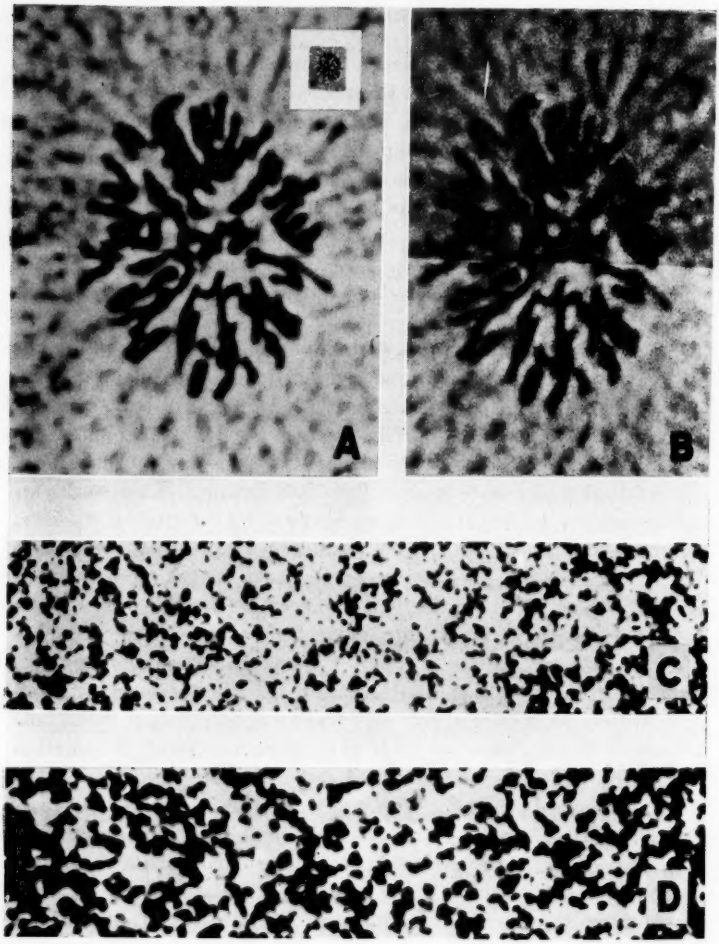

FIG. 1. *A* and *B* are photomicrographs of a transaxial section of a metaphase within a whitefish egg, projected 10 × from 0.65 NA, 200 × negatives. The final magnification is 2,000 ×, the secondary increase being denoted by comparison with the inserted direct print accompanying *A*. The upper part of each is printed darker to show the fibrilla structure of the cytoplasm which is just beyond the resolution of the lens but may be surmised from the record obtained thru the fine grain process shown in *A*. *A* is from a process panchromatic negative processed with Champlin 15 developer. *B* represents a combination of film and developer which is unsatisfactory for secondary resolution. Figs. *C* and *D* compare the emulsions composing the image of the chromosome shown at the bottom of Fig. *A*, but processed in Ultra Fine Grain, with the image of the same chromosome on a film processed in D 72, these being the coarsest and finest grains, respectively, encountered in these tests. The magnification of *C* and *D* is 1000 × and was obtained by photographing the image on the original negatives with the 0.65 NA lens and fine grain processing. Note the large difference in the size of the silver particles.

which obtains for any developer by varying the time and temperature is inversely proportionate to the fineness of grain. The emulsion of process panchromatic films and Wratten M plates is so fine *per se* that contrastive development in such formulae as D 19 and D 72 permits contact printing and mild enlarging with diffusion (ground glass) enlargers without any objectionable graininess. Fine grain developers make the grain of the finished image on fine grain films proportionately finer. Eastman process panchromatic film was used in these tests as a standard in determining the feasibility of different fine grain formulae for photomicrographic purposes.

TABLE 1. TABLE COMPILED FROM STUDY OF SEVERAL NEGATIVES TO SHOW THE UTILITY OF REPRESENTATIVE FINE GRAIN DEVELOPERS FOR PHOTOMICROGRAPHIC PURPOSES*

Developer	Exposure time seconds	Developing time minutes	Temperature of developer	Contrast and tone rating	Practical limit of critical enlargement for 8 mm. lens negative
D 72	2	7	70	Good	2–4 ×
D 76	7	7	70	Excellent	4–5 ×
Edwal 20	10	21	65	Good	4–5 ×
Infinol	9	15	68	Good	4–5 ×
Sease III	10	23	65	Good	4–6 ×
Champlin 15	10	21	70	Excellent	8–12 ×
Eastman Ultra Fine Grain	10	20	65	Poor	10–15 ×
Eastman Ultra Fine Grain	7	40	65	Good	8–10 ×

*These results are based on comparable photographs of a mitotic figure in whitefish eggs. D 72, which is less contrasty than D 19, is included as a standard of comparison. The data for the practical limit of enlarging are based on a study of a large number of negatives. This limit is a function of the initial contrast of the subject and of the resolving power of the emulsion as effected by different developers.

Fine grain principles are easily applied to low magnifications. For high magnifications, it was necessary to discover a developer that might be classed as normally fine grain and normally high contrast. Table 1 shows the result of comparative tests using as photographic subject a transaxial section of the metaphase of a mitotic figure in the egg of a whitefish. To permit further comparison by other workers, a typical slide sold by the General Biological Supply House, Chicago, Ill., under the number E 1378 was used.

The data in Table 1 are based on what may be termed normal development time and temperature for each of the developers.[5]

[5]With the exception of the last figures for Eastman Ultra Fine Grain.

Where the subject has great contrast because of initial constitution or contrasty staining or selective light filtering, any of these developers, including Sease III and Eastman Fine Grain, can be used at these normal times and temperatures. If the final (enlargement) magnification is to be very high, one of those listed as being very fine grain must be used. Fig. 4 shows how negatives developed with representative developers behave when projected to 10 × thru a condenser enlarger.

FIG. 2. *A* and *B* are yeast cells stained in Giemsa stain to show the very small nucleus. *A* is a direct print from 1.40 NA, process panchromatic Champlin processed negative at a projection of 160 mm. *B* is a print from *A* enlarged to 3,000 ×, the magnification scale being 3 mm. = 1 μ. *C* is from a methylene blue preparation photographed with a 3 mm. 1.25 NA lens to show spores and printed to show the area of the spore on the right which represents nuclear content. Emulsion-resolution permits prints which can be accurately measured for comparison of cell parts in difficult subjects such as yeast.

Choice of fine grain developer: Different emulsions respond in different degrees to various fine grain formulae. If vibration of the mechanism or movement within the microscope field forces one to use a fast emulsion, a series of tests is necessary to discover the best formula for the particular film involved. Eastman Panatomic film, for example, altho truly fine grain for macroscopical subjects, cannot be used satisfactorily even with the Champlin formula No. 15, if the film is to be subjected to condenser enlarging at over five times. This film can be so used if the Eastman Ultra Fine Grain developer is employed at a gamma rating of approximately 0.80. The new developer Infinol may prove useful for fast films. This subject will require investigation by each worker to meet his special requirements, especially since new fine grain formulae are continually being developed.

As stated above, any of the fine grain developers mentioned is satisfactory for process panchromatic film where secondary enlargement does not exceed 5 × and initial magnification does not exceed 300

diameters.[6] It is claimed by originators and makers of fine grain formulae that enlargements up to 30 × or even 60 × are possible. Such statements apply to pictorial enlarging only. Critical illumination enlargements show the outlines of individual crystals even in process films when any of these developers is used if the projection exceeds 15 ×. Graininess which would be unnoticed in macroscopic subjects, moreover, becomes very objectionable in photomicrographic records. For convenience, it is very desirable that developing times

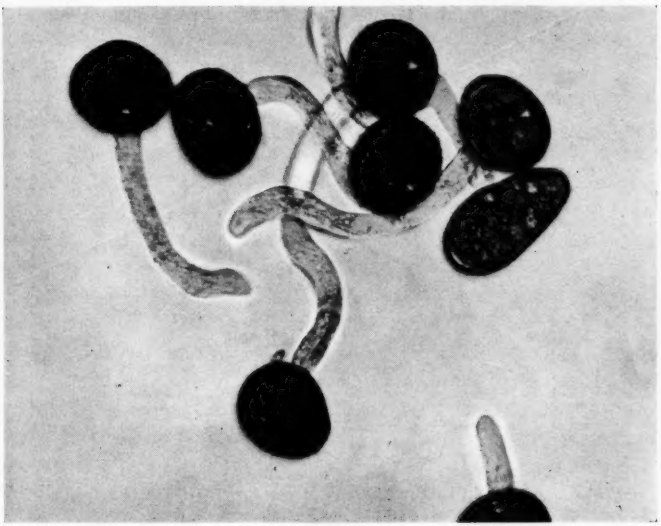

FIG. 3. Urediospores of *Puccinia convolvuli* germinating. The negative was made with an 0.65 lens, and processed in Champlin to show the textural rendering of the hyphae. The original magnification was 200 ×. The print represents a 5 × enlargement from the negative reduced one-half for making this illustration. Final magnification 500 ×.

should not exceed 25 minutes. In our tests, the Champlin No. 15 formula produced the highest contrast and proportionately finest grain[7] in the shortest time. The Eastman Ultra Fine Grain when

[6]The practical limit of secondary enlargement is also affected by the initial magnification which is proportionate to the magnification of the objective and in turn limited by the NA of the objective. The higher the initial magnification the more difficult it is to obtain negatives which will stand great secondary enlargement. For average subjects negatives made with oil immersion lenses should be developed to a fairly high contrast if secondary enlargement is to be attempted.

[7]Champlin, H. 1937. Champlin on fine grain. Camera Craft Pub. Co.

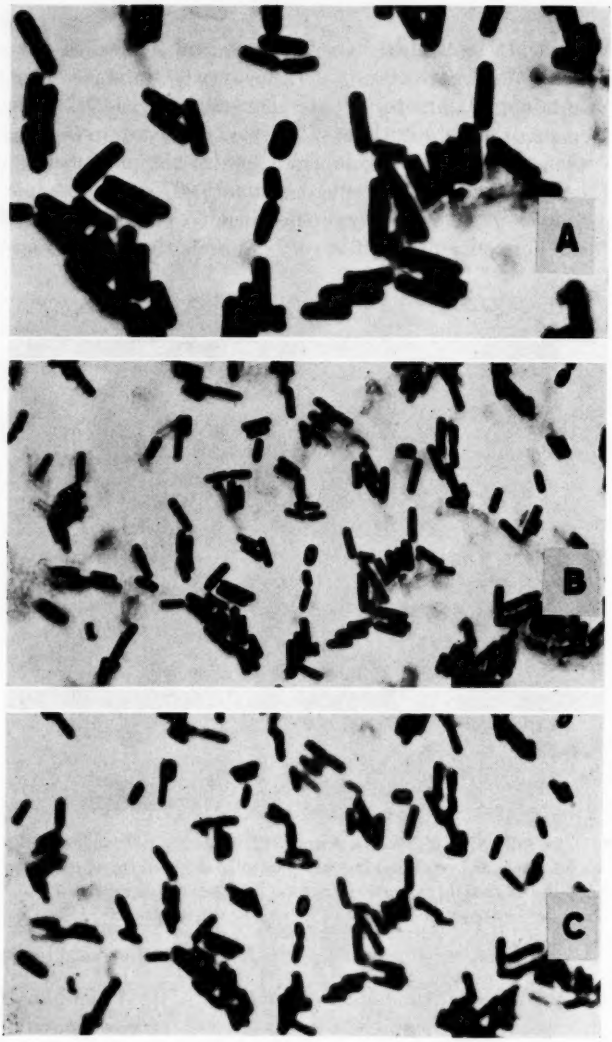

FIG. 4. Photomicrographs of spore bearing *Bacillus prausnitzii* stained with basic fuchsin. *A* and *B* are from a negative made with a 3 mm. 1.40 NA lens at an initial projection of 360 mm. developed to high contrast in D 72. *B* represents a non-critical enlargement of 2½ × bringing the magnification up to 2,000 ×. *A* is a critical enlargement from the same negative increasing the magnification to 5,000 ×. *C* is made with an 8 mm. 0.65 NA lens at an initial projection of 160 mm. and critically enlarged ten times to 2,000 ×. The negative was processed in Champlin 15. Note that *C* compares favorably with *B*, but that *A* is noticeably superior to either of the others. The spore plainly shown in *C* may be considered to be theoretically beyond the resolution of the 0.65 N. A., 8 mm. "dry" lens which was used in making the negative.

used at the top of its gamma curve (30 minutes at 65° F.)[8] develops less contrast than Champlin No. 15 at 20 minutes and then evidences noticeable graininess. Where very high secondary magnification is desired either of these developers may be used to good advantage but with the following difference in choice: The Champlin formula seems best for general subjects, as for example, bacteria, chromosomes, or general views of stained sections; the Ultra Fine Grain developer is excellent for determining the shape of particles which are on the border line of visibility and is very usable at low magnifications where excess contrast should be avoided.

The effect on the emulsion speed is another important point to consider in choosing a fine grain developer. The Champlin formula in our tests is only one-third slower than D 76; that is, the exposure for comparable results need be increased only one-third. Infinol proves faster than Champlin No. 15 but the coarseness of the grain in process panchromatic emulsion makes it inferior for critical work. The other developers are noticeably slower.

Notes on the Champlin formula: For average subjects when using process panchromatic films, we have found the Champlin No. 15 formula to be the most satisfactory for obtaining very fine grain. It does have several objectionable features and unless one is careful in preparing and using the developer very unsatisfactory results may obtain. The detailed discussion in Champlin's book and in his supplementary article[9] should be digested before one attempts to experiment with the formula. For photomicrographic application the following suggestions may be helpful:

(1) The developer must be made up in distilled water. The temperatures suggested by the originator should be followed very closely in preparing the developer.

(2) The exposure for process panchromatic film should be such that the negative assumes good density and strong contrast when developed for 20 minutes at 71° ± 1° in *fresh* developer.

(3) For tray development one may either use a small amount of the developer once or twice and then throw it away or else use a large volume several times and note the lag in rate of development, which accompanies its use. The lag in subsequent development is one of the chief objections to the developer.

(4) One must use extreme care to prevent this developer from touching clothes; it stains badly.

If these precautions are followed, fine grain developers, such as this one, open a new field in photomicrographic work.

[8]Vittum, P. W. and Crabtree, J. T. 1935. A new fine grain developer. Camera Craft, **22**, 587–597.

[9]Champlin, H. 1937. Concerning Champlin 15. Camera Craft, **44**, 426–529.

Other advantages of fine grain: Beginners in photomicrographic work tend to make low power negatives entirely too contrasty. When half-tones are made from such pictures they often fail to show the points involved. If fine grain developing is used, the negative is certain to maintain better tonal values; these can be emphasized as desired in printing.

Fine grain developers are very useful in photographing hyalin objects such as fungus hyphae mounted in water. This use we had not anticipated. Formerly we used D 19 thinking that the highest possible initial contrast would be necessary. The explanation seems to be that the fine grain records the texture of the subject which corresponds to the eye interpretation. A white mycelial thread examined in clear water presents visually a textural difference rather than a color difference. The use of a contrasty developer attempts to exhibit a contrast which does not exist. The fine grain image, being a record of texture, is far more lifelike.

Applications and illustrations: The negatives for the illustrations in this article, except Fig. 2, were made with an 8 mm., 0.65 NA lens. The initial magnification of such a lens is 20 × and the theoretical usable magnification is 650 times. The eyepiece limit would be 33 ×. The accompanying illustrations made with this lens are at magnifications of 1000 to 2000 ×. The result equates using a 50 × eyepiece or even a 100 × eyepiece. Every picture, except where indicated, is made by using a Makam camera, process panchromatic film, Champlin No. 15 developer and a condenser enlarger. As stated in the introduction, no claim is made that such a combination increases theoretical resolution but the following fact may be observed in Fig. 4, C, showing spores of bacteria photographed with the 8 mm. lens at a final magnification of 2,000 ×. This photograph represents an amazing performance for such a lens since these spores are less than 0.5 μ across and the theoretical limit of resolution for the lens with white light is 0.85 μ.[10] Few bacteriologists would dream of using an 8 mm. lens to observe spores in bacteria. These same methods of processing combined with ultraviolet light, quartz objectives, suitable eyepieces, and ideal initial projection distances should permit usable magnifications of 5,000 × to 15,000 ×. Theoretical resolution will not be increased, but usable resolution may, because fine grain processing enables the resolving power of the emulsion to keep pace with the resolving power of the lens system. For average equipment, however, fine grain processing will prove more readily adaptable to intermediate objectives than to the oil immersion series with the exception of the new 4 mm. oil immersion 1.00 NA lens recently issued by an American optical company.

[10]Seifriz, William. 1937. Methods of research on the physical properties of protoplasm. Plant Physiology, 12, 99–116.

IMPROVEMENTS IN THE PERMANENT ROOT TIP SQUASH TECHNIC

B. B. HILLARY, *Department of Botany, University of Toronto, Toronto, Canada*

Corn Syrup Mounts: In a previous paper[1] the author recommended a mixture of acetic acid and corn syrup as a simple and convenient mounting medium for root tip squash preparations. In work carried on since the publication of that paper it has been found with certain samples of corn syrup that the stain loses its brilliance and, in some cases, fades badly. This occurs only with the acetic acid corn syrup mixture and not with a water corn syrup mixture.

The conclusions of the previous paper were based on slides made in October, 1936. These slides are still in perfect condition, and no difference can be detected between those mounted in acetic acid corn syrup and corn syrup diluted with water. A series of very valuable slides, however, made in October, 1938, and mounted in the acetic acid corn syrup mixture, became worthless after two months. In this case a different lot of corn syrup was used; and the same thing has occurred with other samples.

The acetic acid corn syrup mixture was used because the acetic acid hastens penetration of the corn syrup into the cytoplasm and produces at the beginning clearer and sharper mounts, which, for immediate observation, are much better than water syrup mounts. After standing for a day or two, however, there is no observable difference between the two. Therefore, considering the variability of commercial corn syrups and their action with acetic acid, it is advisable, in order to ensure satisfactory results with this mounting medium, to use syrup diluted with water only, and the proportion of 1 part of corn syrup to 1 part of water is recommended.

Dioxan-balsam Mounts: One of the advantages of the corn syrup method is that separation and spreading of the cells is easily carried out in this medium, but this is not always the case with dioxan-balsam, as some root tips resist squashing after dehydration. This is due to the pectic acid, on dehydration, forming a hard compound which cements the cells together. Thus when one attempts to divide the root tip into longitudinal sections by means of needles, it breaks up into irregular pieces which are difficult to squash.

In order to overcome this difficulty it is necessary to remove the

[1]Hillary, B. B. 1938. Permanent preparations from rapid cytological technics. Stain Techn., **13**, 161–7.

pectic substance of the middle lamella. Cormack[2] has shown that the middle lamella below the zone of root hair formation consists mainly of pectic acid. This can be removed by treatment with bases such as NH_4OH and NaOH which convert the pectic acid into a soluble pectate. It was found necessary to apply this treatment before hydrolysis, otherwise a poor stain resulted. Apparently the aldehyde or color producing groups which are liberated by the acid hydrolysis undergo chemical degradation in the presence of a base, thus interfering with the Feulgen reaction.

Various concentrations of NaOH and NH_4OH were tried at different temperatures for varying periods of time. NaOH is not only unreliable but is likely to damage the cell structure seriously and to interfere with the staining. NH_4OH, on the other hand, brings about a clear-cut separation of the cells, without damaging them, and any left in the tissue is easier to remove as the NH_3 volatilizes. Of the different combinations of time, temperature and concentration used, 4% NH_4OH at 60° C. for 15 minutes, then 30 minutes washing, as pretreatment before the application of the Feulgen technic, produced the best preparations.

If it is desired to study the variations in the shape of nuclei which are to be found in the region of elongation, a different means of removing the pectic substance is necessary, since the middle lamella at this region consists of calcium pectate. This can be removed by ammonium oxalate which converts the calcium pectate into calcium oxalate and ammonium pectate, both of which are soluble and easily washed out. Treatment with 4% ammonium oxalate for 40 minutes at 60° C. before hydrolysis will bring about a separation of the cells and will not interfere with the stain. This treatment will also bring about a separation of the cells at the zone of active division, but it is not as rapid nor does it produce as good results as NH_4OH.

The procedure recommended for making permanent dioxan-balsam preparations of root tips is as follows:

1. Fix 30–60 min., preferably in a Navashin type fixative.
2. Wash 30 min. or longer in running water.
3. Treat 15 min. in 4% (by volume) NH_4OH at 60° C. in a corked vial (to prevent evaporation of the ammonia).
4. Wash 30 min. in running water to remove soluble pectate.
5. Hydrolyse 8 min. in *N* HCl at 60° C.
6. Stain 20 min. or longer in fuchsin-sulfurous-acid.
7. Bleach in 3 changes of SO_2-water for at least 10 min. per change.
8. Dehydrate in 3 changes of dioxan of 3 min. each.
9. Place a root tip in a drop of dioxan-balsam on a slide, separate into fine longitudinal strands by means of sharp needles.

[2]Cormack, R. G. H. 1935. Investigations on the development of root hairs. New Phytol., **34**, 30–54.

10. Put on a cover slip, apply pressure to spread the cells and complete their separation by gentle tapping of the cover slip with a needle holder.

The addition of the NH_4OH step to the procedure easily allows one to make from any type of root tip, clear, sharp, and brilliantly stained preparations which show plates of cells one cell thick, filaments of cells, and individual cells.

DIOXAN DEHYDRATION FOR PARAFFIN EMBEDDED FUCUS SLIDES

AUSTIN O. SIMONDS, *Dept. of Botany, Colorado State College, Fort Collins, Colo.*

Good *Fucus* slides are difficult to prepare using the paraffin method, and those available commercially are often poor. Some supply houses ask a small premium price for celloidin prepared slides of *Fucus*. Excellent paraffin preparations can be made, however, using the slow dioxan method[1] and staining with fast green F. C. F. and safranin. (See figure.)

Because of the toxic properties of dioxan, certain precautions are advisable. In the writer's laboratory a large series of bottles is never

FIG. 1. Photomicrograph showing detail of *Fucus* conceptacle prepared using method herein described.

handled at a time and the containers are kept tightly closed as much of the time as possible. This prevents the fumes from escaping into the room for a considerable length of time; and if ordinary precautions for ventilation are taken when containers are opened, it is felt that there is no danger from the use of this reagent.

Fresh plant material was processed in a chromic-acetic-formalin fluid. (Solution A: 1% chromic acid 87 cc., glacial acetic acid 13 cc.; Solution B: comm. formaldehyde 20 cc., water 80 cc.; an equal quantity of each solution was used in the processing fluid.) After

[1]Ralph, Paul. 1938. A comparative study of some dehydrating and clearing agents. Stain Techn. **13**, 9–15.

24 hours in the killing and fixing fluid, the following dehydrating schedule was followed: Dioxan 33% and water 67% for 2 hours; dioxan 67% and water 33% for 2 hours; two changes of 100% dioxan, 1 hour each.

Paraffin infiltration followed McWhorter and Weier's[2] procedure except as to the time the materials were left in the solutions. Following the second change of anhydrous 100% dioxan the pieces of *Fucus* were placed in infiltration solution 1 (50 cc. dioxan, 5 cc. xylol, and 20 cc. of melted paraffin). The paraffin was allowed to "cake" and the liquid beneath the solidified paraffin was allowed to remain on the tissues at room temperature for 4 hours. The pieces of *Fucus* were transferred from solution 1 to infiltration solution 2 (50 cc. dioxan, 10 cc. xylol, 50 cc. melted paraffin). The material was then placed in oven (50° to 52° C.) for 2 hours. The next two changes were to soft paraffin for 2 hours each followed by embedding in paraffin in the usual way.

Sections cut 10μ thick were fixed to the slide and run thru the following staining schedule: 100% xylol; 100% xylol; 95% alcohol; 60% alcohol; water; aqueous safranin[3] (about 0.5%) for ten minutes; water; 50% alcohol; 95% alcohol; fast green F. C. F.[4] (about 0.25% in 66 cc. of 95% alcohol plus 33 cc. of anhydrous acetone). The slides were left in the fast green long enough to differentiate the reproductive organs. Following the fast green, the slides were placed in 100% acetone, then in carbol-xylol, then in 100% xylol, followed by 100% xylol, then balsam and cover glass.

The slides were allowed to remain in each solution down to water for about 5 minutes. The time in the 50% alcohol and 95% alcohol solutions need be only 3 minutes. The dilution of the alcoholic fast green staining solution with acetone slows down the action and makes it easier to use.

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[2]McWhorter, F. P. and Weier, E. 1936. Possible uses of dioxan in botanical micro-technic. Stain Tech. 11, 107–117.

[3]Safranin O, (not certified), from G. G übler and Company, Leipzig, Germany.

[4]Fast green F. C. F., (not certified), from Michigan Biological Supply Company, Ann Arbor, Michigan.

A TRIPLE STAIN FOR AMPHIBIAN EMBRYOS

DANIEL W. SLATER and ERNST J. DORNFELD, *Department of Zoology, Oregon State College, Corvallis, Ore.*

Methods commonly employed for staining early amphibian embryos are in certain respects inadequate. With iron hematoxylin it is notably difficult to stain nuclei to the proper intensity without at the same time overstaining the yolk granules. In addition, most counterstains generally used after Delafield's or similar hematoxylins, fail to produce color differentiation between yolk granules and ground cytoplasm. In attempting to stain salamander embryos (*Triturus*), the writers have found a simple tri-stain combination which yields the desired cytological distinctions without loss of clarity in the embryological configuration. This follows the tendency emphasized by Gatenby[1]—"a trend away from these older techniques towards the use of certain methods which give more information about the histology and cytology of development".

The stain combination we suggest involves the successive use of Harris' hematoxylin, safranin, and fast green. The following schedule has been found to be most effective:

1. Fix embryos for six hours or longer with the Bouin-dioxan mixture of Puckett[2] which consists of two parts Bouin's fluid and one part dioxan. (This mixture avoids the shrinkage and hardening usually encountered after other fixatives. Good staining results were also obtained after Zenker, but brittling and shrinkage occurred.)

2. Wash and dehydrate with dioxan; clear for five minutes in xylol to hasten infiltration; infiltrate and imbed in hard paraffin (56–58 C.). Section and mount as usual.

3. Stain for five minutes in Harris' hematoxylin[3] (National Aniline Co., FH-12) and wash in water. Destain with 35% acid alcohol and blue in tap water.

4. Stain for five minutes in 1% safranin O (National Aniline Co., NS-10) made up in aniline water, and wash in tap water.

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[1]Gatenby, I. B. 1928. *In* Lee's Microtomist's Vade-mecum, *9th Ed.*, 279. P. Blakiston's Son & Co., Philadelphia, Pa.

[2]Puckett, William O. 1937. The dioxan paraffin technic for sectioning frog eggs. Stain Techn., **12**, 97–98.

[3]For reference purposes the manufacturer's name and the certification numbers of the batches of stains employed by the writers are given here.

5. Counterstain for one to two minutes with 0.5% fast green (National Aniline Co., NGf-3) made up in 95% alcohol. This step is critical. Control timing so that yolk granules remain bright red while ground cytoplasm is stained green. Wash and dehydrate with absolute alcohol.

6. Clear with oil of cloves or xylol and mount in balsam.

A successfully stained preparation shows the resting nuclei blue against the green of the ground cytoplasm. Chromosomes in mitosis appear purplish and the spindle fibers are sharply defined in green. Yolk granules and nucleoli are red; therefore the former will contrast nicely against the ground cytoplasm. All components are well balanced in intensity and sharply defined one from another. The stains used in this combination also possess the advantage of permanency.

THE USE OF BISMARCK BROWN Y IN SOME NEW STAIN SCHEDULES[1]

GLENN W. BLAYDES, *Ohio State University, Columbus, O.*

ABSTRACT.—**The staining quality of Bismarck brown Y may be improved and sterility maintained by adding 5% phenol to a 1% aqueous solution. Use the phenolic Bismarck brown in combination with iron alum hematoxylin except for stripped epidermis in the following procedures:**

Stem and Root Schedule: Mordant sections from water in 4% iron alum for 10 minutes. Rinse in distilled water and stain in 0.5% aqueous hematoxylin for 1 minute or until darkly stained. Rinse in distilled water and destain in 2% iron alum until a gray color appears. Rinse thoroly in distilled water and intensify hematoxylin by transferring sections to 0.5% aqueous lithium carbonate until the desired black color appears. Rinse thoroly in distilled water and stain for 1–5 minutes in phenolic Bismarck brown. Rinse in distilled water, dehydrate successively in 30, 50, 70, 95 and 100% alcohol. Clear in methyl salicylate for 5 minutes, then to xylene for 3–5 minutes, and mount in balsam.

Middle Lamellae in Wood: Destain more thoroly in 2% iron alum than for the general stem and root schedule, and intensify in lithium carbonate for a longer period (about 1 hour).

White Potato Tuber Sections: Modify above schedule by reducing time of destaining in 2% iron alum to about 30–60 seconds and intensify hematoxylin until starch grains appear bluish in color. Stain in phenolic Bismarck brown for 1–2 minutes.

Wheat Grain Sections: Fix grain for sectioning when in "dough" stage. Use schedule the same as for potato tuber except for reducing time of staining in phenolic Bismarck brown to about 45 seconds.

Tradescantia zebrina Epidermis: Strip epidermis from leaf while submerged in water. Fix in 100% alcohol 10 minutes, pass thru 95, 70, 50, 30, and 10% alcohol to water. Stain in phenolic Bismarck brown for 10–20 minutes. Dehydrate, clear in methyl salicylate and mount in balsam.

Bismarck brown Y (anilin brown) merits a wider use as a plant tissue stain. Harrar (1928) has recommended Bismarck brown for phloem. It is also useful for staining many other tissues. In combination with iron alum hematoxylin, clear differentiation is obtained

[1]Papers from the Department of Botany, The Ohio State University, No. 417.

and the stains are relatively permanent. Tissues stained with this combination photograph quite satisfactorily. As a plant histological stain, Bismarck brown Y[2] produces better results in an aqueous solution than in alcohol; but after an aqueous solution has stood for a week or ten days at ordinary laboratory temperatures a fungus often develops in it, necessitating frequent filtering. In an effort to prevent growth of this fungus and thereby eliminate the need of frequent filtering, alcohol, toluene, formalin and phenol were added in various concentrations, as antiseptics, and tests were made as to the staining properties as compared to the dye when made up in distilled water. Transverse sections of *Menispermum canadense* stems were used as test material. All sections were cut 26 μ in thickness and carried thru the same stain schedule. It was found that alcoholic solutions as dilute as 20% were effective in preventing growth of the fungus, but the stain produced was inferior to that produced by other solutions. Four drops of toluene added to 50 cc. of an aqueous solution of Bismarck brown kept it sterile and the staining reaction appeared to be the same as for the pure aqueous solution. When the dye was made up in 5% formalin, growth of the fungus was prevented and a superior staining reaction resulted. The same was true for 5% phenol solution. The phenol solution was regarded as being somewhat superior to the formalin solution in differentiation and definition. This stain solution is referred to in the following schedules as *phenolic Bismarck brown Y*. The formula is as follows:

Bismarck brown Y (anilin brown Y).....	1 gram
Phenol crystals.....	5 grams
Distilled water.....	100 cc.

Mix the constituents, allow to stand for 1 hour, then filter. The solution is then ready for use and will keep indefinitely. Recently the writer discovered that Simmons (1886) over 50 years ago used carbolic acid in Bismarck brown as a stain for leaf tissues, epithelial cells and bacteria.

Hematoxylin with Phenolic Bismarck Brown Y for Herbaceous and Woody Stems and Roots

1. If sections are in 95% alcohol transfer at 3 minute intervals through 70, 50, and 30% alcohol to distilled water.

[2]The original experiments with Bismarck brown Y were made in 1933 with uncertified dye produced by Coleman and Bell, Norwood, O. More recently certified Bismarck brown Y, No. CN 1 and CN 3, produced by the same manufacturers have produced almost identical results. Dr. Elizabeth Wagner aided the writer in some of the original experiments.

2. Transfer sections to 4% aqueous solution of iron alum (ferric ammonium sulfate) for 10 minutes.

3. Rinse with 6 changes of distilled water to remove excess mordant (iron alum).

4. Decant the water and cover sections with a 0.5% aqueous solution of hematoxylin[3] for 1 minute or until they are dark colored.

5. Rinse in 2 changes of distilled water to remove excess hematoxylin.

6. Decant the water and cover sections with a 2% solution of iron alum. This destains and removes undesirable hematoxylin precipitate. Permit the destaining to continue until the tissues have a grayish appearance. A few trials likely will be necessary before the right point is reached.

7. To stop the destaining action decant the iron alum and rinse 6–8 times in distilled water.

8. Intensify the remaining hematoxylin by transferring the sections to a 0.5% aqueous solution of lithium carbonate.[4] The time required ranges from a few seconds to an hour, depending upon the amount of hematoxylin remaining on the cell walls and the intensity desired. The black color produced by action of the lithium carbonate on the hematoxylin is quite desirable because of the increased differentiation.

9. Rinse in distilled water 2–3 times to remove excess lithium carbonate. If this is not thoroly removed, a flocculent precipitate appears when the sections are placed in the phenolic Bismarck brown.

10. Decant the water and cover the sections with phenolic Bismarck brown Y. The time of staining varies with different structures and the degree of intensity preferred. Usually from 1–5 minutes is sufficient.

11. To stop action of the phenolic Bismarck brown Y decant the stain and rinse the sections with distilled water.

12. Decant the water and dehydrate the sections by an alcohol series consisting of 30, 50, 70, 95 and 100% at 3 minute intervals. If the tissues are imbedded in collodion, the 100% alcohol may be dispensed with by the use of ether-absolute-alcohol (half and half

[3]Camp's (1930) method of preparing aqueous hematoxylin is very satisfactory. Distilled water is brought to the boiling point. The heat source is removed, then the hematoxylin (light) crystals are added. The solution is ready for use as soon as it is cool. A trace of sodium bicarbonate added to the solution improves the immediate staining qualities.

[4]A 0.5% lithium carbonate solution in distilled water is an excellent reagent for intensifying hematoxylin in many tissues. For some species, however, it has a macerating effect on cortical tissues. This has been noted for young and mature stems of *Helianthus annuus*, 3–5 year old stems of *Asimina triloba*, *Saururus cernuus* stems and the fruiting tips of *Fucus vesiculosus*. Sometimes the macerating effect may be removed by diluting the lithium carbonate. This dilution, of course, slows up the intensifying action on the hematoxylin. Sodium carbonate, used in the same way as the lithium, occasionally has the same macerating effect. If these solutions cannot be used, alkaline tap water or dilute ammonia may be employed.

mixture) which will dehydrate the tissues and remove the collodion at the same time. Make 2–3 changes.

13. Transfer the sections to oil of wintergreen (methyl salicylate) for 5 minutes.

14. Transfer the sections to xylene for 5 minutes or longer, then mount them in Canada balsam.

Staining Schedule for Middle Lamellae in Wood

Excellent preparations demonstrating middle lamellae may be made from fresh or dried wood of many species. *Asimina triloba* and *Pinus strobus* are very satisfactory forms with which to work. Small wood blocks stored for 1–3 weeks in glycerine with 95% alcohol (half and half) will section satisfactorily without imbedding, on a sliding microtome. Air in the wood blocks should be removed by pumping when first placed in the glycerine-alcohol mixture, or by boiling in water for a few minutes, then plunging in cold water, repeating this process until sinking occurs. Then boil again and plunge the blocks immediately in the glycerine-alcohol mixture. The blocks may be left in this preservative indefinitely. After 2–3 years, however, white pine shows some deterioration. Occasionally cells may have the primary and secondary wall pulled loose from bordering lamellae.

The *hematoxylin phenolic Bismarck brown Y schedule for stems and roots*, with the following modifications, is excellent for sections of wood to demonstrate middle lamellae:

1. Destain more thoroly with the 2% iron alum solution, or until a light gray color is obtained.
2. Rinse in distilled water 6 times.
3. Transfer to 0.5% lithium carbonate solution for 30–60 minutes, or until the middle lamellae appear as black lines.
4. Continue as for the regular stem and root schedule.

A Modified Staining Schedule for White Potato Tuber Sections

The hematoxylin phenolic Bismarck brown Y combination is especially good for differentiating starch grains, protein crystals and internal phloem in the white potato (*Solanum tuberosum*) tuber sections. When properly stained the starch grains will show a bluish color similar to that produced by iodine. The striations and hilum are satisfactorily differentiated. Cubical protein crystals may be found in cells a short distance (3–5 cells) inside the cork layer. With the following staining schedule these crystals stain light brown to a red brown color. Only occasional tubers have an abundance of these crystals. Potato tuber pieces will imbed and section satis-

factorily from collodion, using Wetmore's (1932) method. For staining, modify the stem and root schedule as follows:

1. Destain slightly in the 2% iron alum, 30–60 seconds usually being sufficient. The sections after destaining should have a dark gray color.
2. Intensify the hematoxylin with 0.5% lithium carbonate solution until the starch grains show a bluish color.
3. Stain in phenolic Bismarck brown for 1–2 minutes.
4. Finish as in steps 12, 13 and 14 of the *stem and root schedule*.

Stain Schedule for Wheat Grains

Wheat grains for sectioning should be collected when in the "dough" stage. With a sharp razor blade slice off a tip of each grain to aid entrance of the fixative. Fix in a chromo-acetic acid solution using Schaffner's, Nawaschin's or the Chicago formula. Removal of air with a filter pump aids in better fixation. Wheat grains prepared in this way may be imbedded in collodion and sectioned satisfactorily. Wetmore's (1932) hot collodion method is very satisfactory. Use the same schedule as for potato tubers except that care must be taken *not* to overstain in the phenolic Bismarck brown. Usually 45 seconds is sufficient time for this stain. When properly done the very delicate endosperm cell walls will appear as fine brown lines, the starch grains a bluish black color, irregular dark masses of protein may occur along with the starch grains, aleurone cells will be filled with brown protein granules and the nuclei are stained dark blue.

Preparation and Staining of Epidermal Tissue for Permanent Mounts

1. Allow *Tradescantia zebrina* (Wandering Jew) leaves to wilt for 4–6 hours.
2. Strip off epidermal layers, keeping the leaf submerged in water during the process. This prevents serious rolling of the tissue.
3. Fix the strips in absolute alcohol for 10 minutes.
4. Transfer at 3 minute intervals through 95, 70, 50, 30 and 10% alcohol, then into distilled water.
5. Stain in phenolic Bismarck brown for 10–20 minutes.
6. Rinse in distilled water.
7. Dehydrate at 3 minute intervals in 10, 30, 50, 70, 95 and 100% alcohol.
8. Transfer to oil of wintergreen (methyl salicylate) for 10 minutes or longer.
9. Cut the strips into ⅛ inch squares with a sharp safety razor blade.
10. Transfer to a drop of balsam on a slide, being careful to carry over as little wintergreen oil as possible.
11. Examine with a microscope to see that the outer surface of the epidermis is turned up. Then cover with a cover-glass.

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LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

BOOK REVIEWS

BENSLEY, R. R. and BENSLEY, S. H. **Handbook of Histological and Cytological Technique.** 8½ x 11 in., 167 pp. Cloth, with ring binding. Univ. of Chicago Press, Chicago, Ill. 1938. $2.00.

This book is the outgrowth of the authors' laboratory instruction leaflets, and the material given in it has previously been available in mimeographed form. Probably it will prove of limited usefulness in other than the authors' own classes. It contains 30 pp. of "Techniques for the study of fresh tissues" and about 130 on "Techniques for the study of fixed tissues." Unfortunately formulae for staining fluids and other solutions are often indefinite; it is not specified whether chemicals are to be used in crystaline or anhydrous form, dye-content of the stains called for is not mentioned, and only in a few instances are the sources given from which the dyes employed by the authors were obtained.—H. J. Conn.

STITT, E. R. CLOUGH, P. W. and CLOUGH, MILDRED C. **Practical Bacteriology, Haematology and Animal Parasitology.** *9th Ed.* 6 x 8½ in., 961 pp. Cloth. 208 illustrations. P. Blakiston's Son and Co., Philadelphia, Pa. 1938. $7.00.

One must take the authors' word that this is "9th edition", altho the book comes out under a new title, with additional authors, in a new format, and is completely rewritten. In other words, it is virtually a new book, 124 pages longer than the 8th edition. For bibliographic purposes, the change from the old title is a little unfortunate; the 8th edition was "Practical Bacteriology, Blood work and Animal Parasitology" by E. R. Stitt. The new edition is improved in appearance because of wider margins; this makes the page larger, altho the typepage has not been changed in size.

This book is too well known to need many words of description. It is especially to be noted, however, that the material on apparatus, culture media and staining methods, formerly comprising the first three chapters of the book, have now been transferred to the appendix. This appendix is now over 100 pages long and constitutes a concise manual of bacteriological methods. The fact that methods of staining and preparing culture media have been transferred to it does not mean that they have been slighted by the authors. On the contrary the formulae have in many cases been brought up to date, the less useful methods omitted and more workable procedures inserted. A table of the solubilities of bacteriological stains is included.—H. J. Conn.

MICROSCOPE AND OTHER APPARATUS

BOETGEHOLD, H. **An improvement of the field of view in microscope objectives.** *Zts. wiss. Mikr.*, 55, 17–25, 1938.

By the use of suitable menisci the author attempts to correct for the curvature of the field of view as is corrected by a homal in photography. The refractive index of such a lens should be positive or only slightly negative. For an objective of medium power a single thick meniscus at the top is sufficient. Its concavity is far greater than its convexity, and is directed away from the object. For a higher power objective a smaller but stronger lens is used at the lower end, its concavity toward the object.

Since these menisci have the chromatic error of converging lenses, a series is used at the top of the high power objective. This requires the use of compensating oculars.

Planachromatic lenses for oil immersion can be made of 1.00 N.A.; with proper choice of dye and use of monochromatic light this becomes equivalent to 1.25 N.A.—*E. Barasch.*

HANSEN, W. W. **On a new design of micromanipulator.** *J. Royal Micro. Soc.*, **58**, 250–2. 1938.

This plan relates to the development of a new type of micromanipulator capable of allowing motion of a quartz needle, or other fine tool, for small distances and in any direction. The conditions attained are: considerable ratio between the motion of the operator's hand and the needle, good rigidity, freedom from backlash, freedom from wear, and ease of construction (no close fits required). On a test for possible lost motion it is found that the average difference in micrometer settings amounts to about one wave length of light.—*J. A. de Tomasi.*

MACNEAL, W. J. and BLEVINS, A. **A culture slide for dark-field microscopy.** *Science*, **88**, 554. 1938.

The slide (Arthur H. Thomas Co., Philadelphia, Penn.) has a central circular stage about 3 mm. in diameter which is surrounded by a circular moat approximately 0.9 mm. deep and 4.5 mm. wide at the top. The wall slopes away from the central stage so that the stage is at the upper surface of a truncated cone with a broad base. A second narrow and shallow groove entirely encircles the moat, slightly separated from it. The former is used for the culture medium, the smaller groove catches the excess vaseline or paraffin used for sealing the cover slip to the slide.—*J. A. de Tomasi.*

MAYNARD, C. W. **An efficient paraffin bath.** *Am. Jour. Clin. Path., Tech. Suppl.* **3**(2):73–75. 2 figs. 1939.

Lamps, wired in parallel, dip into drinking glasses containing paraffin and paraffin diluted with chloroform. Heating is controlled by using appropriate size bulbs. Tissues in perforated containers rest on the unmelted paraffin. Here the temperature cannot be much above the melting point of the paraffin.—*G. H. Chapman.*

MILLS, JOHN H. **A multiple ring maker for the Kline test.** *J. Lab. & Clin. Med.*, **24**, 189–191. 1938.

The Kline test is a microscopic precipitation test for laboratory diagnosis of syphilis, performed by mixing 0.5 cc. of heated serum and about .008 cc. antigen emulsion on a glass slide and observing with 100–120 times magnification. Usually four rings are made for each test: unknown serum plus diagnostic antigen; unknown serum plus exclusion antigen; known positive serum; known negative serum.

A rigid brass multiple ring maker is suggested to reduce time required setting up the Kline test. Cut 12 one-inch lengths of brass tubing 16 mm. outside diameter and about 1.2 mm. wall thickness. Solder these to a brass plate, about $\frac{1}{8}$ inch thickness, cut to same dimensions as slides used for test. Space is saved if rows are somewhat staggered. Dress ends of tubes with a file and polish on glass surface sprinkled with emery powder. A bakelite valve handle may be fastened to the top of the brass plate by a screw, making an instrument resembling a rubber stamp. Small vents are drilled thru the plate at the center of each tube. Further helpful suggestions are made regarding the use of this instrument in Kline diagnostic and exclusion tests.—*P. R. Beamer.*

STUDNIČKA, F. K. **A container for the staining of microtome sections placed on glass.** *Zts. wiss. Mikr.*, **55**, 43–44. 1938.

In order to accommodate slides of other than the usual size of 2.6×7.6 cm and more of them without injuring freshly prepared sections to be stained and washed, the author devised the following vessels: (1) A crystallizing beaker, diam. 18 cm., with a vertical wall at least 7.6 cm. high (i.e., length of slides). (2) Another beaker to be placed inside it, diam. 15 cm., perpendicular walls 7.6 cm. high. (3) A third beaker of any height to cover the first two like a lid.

Place the slides in the staining fluid between (1) and (2), the section facing the concavity of the outer wall, and other slides in water in the central beaker for washing.—*E. Barasch.*

PHOTOMICROGRAPHY

WOLF, J. **A new method of marking certain areas in a section.** *Zts. wiss. Mikr.*, **55**, 44–48. 1938.

A method of placing an easily recognizable, easily removable mark on the area of a section to be photographed without injuring the slide or impeding its examination has been proposed by the author. A transparent tape with a cross mark is placed on the cover glass. It consists of (1) transparent, sticky Durex cellulose tape, 19 mm. wide, (Durex Abrasives Corporations, 82 Beaver St., New York), the sticky layer consisting of gum and resin; (2) thin (0.14 mm.), transparent, cellulose tape, 24 mm. wide, with circular holes, diam. 10–12 mm., 5–6 mm. apart; (3) an ordinary thick, square or round cover glass, 18–20 mm. wide, bearing two diagonal lines, drawn with a lithographic pen, crossing in the center of the cover slip.

Place tape (1) on tape (2), leaving only the perforations sticky. Place a cover slip on each 3rd perforation; divide the tape, leaving one marked cover slip in the center and one perforation on each side. Place cross mark on the area to be studied and press it down on sticky holes holding it in place by a clip. Find area in field and focus for photography. Remove tape.—*E. Barasch.*

WYCHERLEY, S. R. **Photomicrography and record photography with Dufaycolor.** *J. Royal Micro. Soc.*, **58**, 244–9. 1938.

After a general discussion of the several color materials available at the present for photographic purposes, this paper describes the nature of the film in the Dufaycolor process, in which the so-called reseau, or color mosaic, is an integral part of the sensitive emulsion. It is pointed out that the fineness of ruling (40 lines to the mm.), together with the high order of transparency of the dyes used, combine to produce a medium peculiarly suited to the needs of scientific photomicrography. The transparency of the film gives ready-made lantern slides. A set-up for this technic, as suggested, may require: 5-amp. white arc at 24 in. from substage, 6-in.-focus collecting lens, Dufay compensating filter, Abbe condenser, ¼ in. objective (N.A. 0.8, W.A. approx. 0.5), × 8 eyepiece. The magnification is × 200, the exposure for a medium-stained specimen 1/10 sec. Full instructions for processing the film are also appended.—*J. A. de Tomasi.*

MICROTECHNIC IN GENERAL

AUMONIER, F. J. **Notes on the distortion of paraffin sections.** *J. Royal Micro. Soc.*, **58**, 253–7. 1938.

Graphic reconstruction of skull development from paraffin ribbons has shown a considerable degree of distortion of serial sections even after spreading. The question which the author seeks to answer is: do the sections, always creased and wavy, regain their full length? Of the many factors that have a bearing on this problem the following are studied: consistency (and melting point) of paraffin, shape of block, sharpness of knife, and angle of blade. It is found, by comparing paraffins of 52° and 58° C. m. p., that low melting point paraffin is preferable. The sharpness of the blade is the limiting factor, i. e. it must be given the greatest care. Variations in the angle of the blade between 5° and 17° do not seem to cause appreciable difference of distortion. As distortion in paraffin is permanent, it is suggested that, whenever possible, celloidin, or double imbedding should be used.—*J. A. de Tomasi.*

GARVIN, T. **The use of dioxan in histologic technique.** *Amer. J. Clin. Path., Tech. Suppl.*, **2**, 175–8. 1938.

An outline of the history, chemistry and toxicology, and use of dioxan. Fix small fragments, over a low flame until vapors arise, in the following solution: 40% formalin, 10 cc.; dist. water, 90 cc. Fix larger sections not more than 2 mm. thick 1–2 hr. at 60–65° C. Transfer to the following baths: 95% alc., 1 hr.; pure dioxan, 2 changes of 1 hr. each; dioxan-paraffin (1:1), 1 hr.; paraffin, 2 changes of 15–30 min. each. Embed, cut, mount and stain as with paraffin.—*G. H. Chapman.*

PORTER, C. L. **Bound water as an aid in the preparation of microscopic mounts.** *Turtox News*, **17**, 14. 1939.

Methods of mounting microscopical preparations which do not lend themselves to the balsam technic (e. g., glycerin jelly or Venetian turpentine) often fail to yield permanent or reliable mounts. The behavior of gelatin, which retains moisture indefinitely in the presence of formalin, has suggested the following mounting technic: Transfer material from water to a very dilute gelatin. Allow gradual concentration until a fairly firm jelly is formed (the slower the process, the less tendency there will be for the material to plasmolyze). Transfer a small portion of the jelly containing the material to a glass slide, warm gently until it melts, and apply a cover slip. Place 1–2 drops of formalin at the edge of the cover slip; let stand several hours to allow diffusion of the formalin; blot and wipe dry. Ring the mount with gold size, lacquer, or varnish.—*J. A. de Tomasi.*

DYES AND THEIR BIOLOGICAL USES

ABRAHAMSON, E. M. **The determination of blood glucose with 2,6 dichlorphenolindophenol.** *Am. Jour. Clin. Path., Tech. Suppl.*, **3**, 60–4. 1 Fig. 1939.

The Folin-Wu filtrate is reduced by ferricyanide in alkaline soln., acidified, and the ferrocyanide titrated with 2,6 dichlorphenolindophenol using a soln. prepared from a standard tablet marketed by Hoffman-LaRoche. This indicator is added from a burette until the pink color of the dye persists.—*G. H. Chapman.*

JOHNS, C. K. **Place of the methylene blue and resazurin reduction tests in a milk control program.** *Amer. J. Pub. Health*, **29**, 239–47. 1939.

For routine testing the methylene blue reduction test has many advantages over the usual counting methods. Its accuracy as an index of the initial bacterial content of the better grades of milk is greatly improved when the organisms are redispersed by inversion of the tubes every 2 hours. The resazurin 1-hour test does not indicate the bacterial content as reliably as does the modified (tube inversion) methylene blue reduction test, the former placing a larger proportion of high count milks in the highest grade. When incubation is continued to the pink stage, the accuracy of the resazurin test compares favorably with that of the methylene blue test, while the reduction time is shortened. The correlation between resazurin color and cell count is much poorer with market milks than with individual quarter samples. Many market milks with high cell counts fail to bring about a significant color change.—*M. W. Jennison.*

PEARLMAN, M. D., and LIMARZI, L. R. **Correlation studies of basophilic aggregation and reticulocytes in various clinical conditions.** *Amer. J. Clin. Path.*, **8**, 608–19. 1938.

The basophilic aggregation test of McCord is shown to be another, but inferior, method for staining reticulocytes and is, therefore, an index of marrow response rather than an indication of lead absorption.—*G. H. Chapman.*

TOBIE, W. C. **Improved procedures in determination of aldehydes in distilled alcoholic liquors with Schiff's reagent.** *Food Res.*, **3**, 499–504. 1938.

The writer suggests certain refinements in technic to avoid some of the difficulties experienced in using Schiff's fuchsin-sulfurous-acid reagent for the colorimetric determination of aldehydes. An entirely colorless reagent may be prepared by treatment with decolorizing carbon: Dissolve 0.500 g. basic fuchsin in 500 cc. warm water, and pass in sulfur dioxide gas until the weight has increased by 5 g. Make up to 1 litre. If still yellow or orange in color, add 1 g. of decolorizing carbon, agitate, and filter with a watch glass covering the filter. Repeat this treatment if any discoloration remains after first treatment. This reagent may be kept 5 months or more in a well-stoppered container. An adjustment for sensitivity is frequently necessary because of variations in dye content of different lots of fuchsin; the solution should show a distinct, altho light, pink color with 0.20 cc. of the dilute standard acetaldehyde as given in the A.O.A.C. method, while the aldehyde-free check is colorless or nearly so. If this does not prove to be the case, adjustment can be made by passing in a little more SO_2 or dissolving in the solution small quantities (e.g. 0.05 g.) of basic fuchsin, until the desired sensitivity is reached. The writer also discusses the preparation of

aldehyde-free alcohol and describes methods of conducting the determination.—*H. J. Conn.*

ANIMAL MICROTECHNIC

BEYER, E. M. **Double imbedding method for rubber paraffin.** *Amer. J. Clin. Path., Tech. Suppl.*, **2**, 173–5. 1938.

The method is useful for blood vessels, endocrine glands, gastro-intestinal tract and for large sections. It permits cutting 5 μ sections without fraying. Heveatex, a natural rubber latex, is spread in a thin film on a glass slide. After drying 24 hr. it is pulled off and cut into small pieces. To 100 g. of paraffin add 2 g. of rubber and 0.5 g. of beeswax. Heat to 105° C. for 16 hr. with occasional stirring. Avoid overheating.

For visceral and neural tissue, employ the following schedule: 10% formalin, 24 hr.; 80% alc., 24 hr.; 95% alc., 24 hr.; abs. alc., 24 hr.; abs. alc. and ether, 24 hr.; 4% celloidin, 24 hr.; abs. alc. and ether, 1 hr.; xylol, 15–30 min. or until transparent then 3 changes of 56–58° C. rubber paraffin in oven for 1½–3 hr. Embed in rubber paraffin as with paraffin.—*G. H. Chapman.*

CROSSMON, GERMAIN C. **Separation of the acidophilic elements of the tissues into two groups.** *Anat. Rec.*, **73**, 163–170. 1939.

Zenker-fixed sections stained with acid fuchsin and immersed in an aqueous phosphotungstic or phosphomolybdic acid solution exhibit two classes of tissue elements: Class 1, those which retain the acid fuchsin (cytoplasm, muscle, erythrocytes, zymogen, etc.); Class 2, those decolorized in the solution (collagen, reticulum, cartilage, mucin, etc.). Class 2 structures are those elements which ordinarily are stained blue in Mallory's connective tissue stain.

Fluorescein dyes may be used instead of acid fuchsin previous to immersion in the acid solution. Class 1 elements then retain a red color; Class 2 elements are orange. Class 2 elements are subsequently decolorized completely in abs. alcohol. They are then generally remordanted in phosphotungstic acid and stained in light green or anilin blue and then differentiated in 1% acetic acid.

This gives three classes or elements ordinarily taking acid dyes. Class 1A (red): erythrocytes, acidophilic granules, zymogen, keratinized epithelium, etc.; Class 1B (lightly stained green or blue): general cytoplasm, smooth and skeletal muscle; Class 2 (green or blue): collagen, reticulum, cartilage, bone, etc. Fifteen steps in making the staining solutions and applying the stain are given in the paper.—*S. I. Kornhauser.*

FAZIO, C. **Betrachtungen über die histologischen Methoden mit Benzidin im Zentralnervensystem.** *Zts. gesam. Neurol. u. Psychiat.*, **164**, 678–686. 1938.

The effect of post mortem changes on Pickworth's benzidine method for blood capillaries (*J. Anat.* **69**, 62–71, 1934; *Abs., Stain Tech.* **11**, 132, 1936) was studied. Rabbits, white mice, guinea pigs, and dogs were used. The author concludes that the method gives valid results up to 48 hr. post mortem in material stored at room temperature or to 5 days when stored in a refrigerator.—*H. A. Davenport.*

GERMAN, W. M. **Hortega's silver impregnation technique, uses and application.** *Amer. J. Clin. Path., Tech. Suppl.*, **2**, 165–71. 1938.

An introduction to Hortega's methods, including general directions, and details for Hortega's general nuclear stain, and the stain for epithelial fibrils, epithelial bridges and protoplasmic reticulum. No new methods given.—*G. H. Chapman.*

GERMAN, Wm. M. **Hortega's silver impregnation stains.** *Am. Jour. Clin. Path., Tech. Suppl.*, **3**, 47–55. 4 Figs. 1939.

Detailed instructions are given for: (a) Hortega's neuroglia stain, which provides an excellent contrast between black stained neuroglia cells and other tissues, stained brown or mahogany. Microglia fibrils are not stained; (b) Hortega's stain for microglia which colors them brown or mahogany red. They can be recognized by a polymorphic nucleus, an irregular cell body and cell processes; (c) Hortega's impregnation method for certain epithelial neurofibrils and cell processes not demonstrated by ordinary methods; and (d) Hortega's modification of the silver tannate method of Achucarro, which demonstrates mitochondria,

cellular fibrils and certain cell processes not seen by ordinary methods.—*G. H. Chapman.*

GERMAN, W. M. **Hortega's silver impregnation methods. Technique and applications.** *Amer. J. Clin. Path., Tech. Suppl.*, **3**, 13–9. 1939.

Details are given for the double impregnation method for connective tissue, which is useful for demonstration of some of the finer connective tissue fibrils in connective tissue, inflammatory tissue, sarcomata of fibroblastic origin, and the fibrillar structure of nerve sheaths. The fibrils stain deep brown to black while epithelial cells stain red or golden brown. The double impregnation method with $KMnO_4$ is useful for staining all connective tissue elements, particularly the finer fibrils which are not seen by ordinary methods. It demonstrates nerve sheath structures and neurofibrils. Gallego's general tissue stain colors nuclei, magenta; epithelial cytoplasm, yellowish pink; connective tissue, brilliant green; muscle, olive green; and cornified epithelium and blood, grass green. Gallego's method for differential staining of elastic tissue gives excellent differentiation between elastic and collagenous connective tissue and is useful for photomicrography. The former stains a brilliant fuchsin red, while the latter stains a brilliant green.—*G. H. Chapman.*

HUMPHREYS, S. P. **A method for the impregnation of perivascular nerves on intracerebral blood vessels.** *Am. J. Path.*, **15**, 151–153. 1939.

Method A. Human material previously fixed in formalin. Dissect out the vessels and wash 12 hr. in dist. water. Soak in the following mixture 4 hr.: 95% alcohol, 90 cc.; glacial acetic acid, 5 cc.; formalin, 5 cc. Wash 2 hr. in dist. water. Impregnate 6 to 24 hr. in Bodian's silver nitrate copper solution—1 to 2% aq. protargol, (Winthrop Chemical Co., New York City) 20 cc.+2 g. metallic Cu (Merck). Wash in 3 changes of dist. water. Reduce with: hydroquinone 5 g., sodium sulfite, 10 g., dist. water 100 cc. Tone in 1% yellow $AuCl_3$ solution, wash in 2 changes of dist. water, treat with 2% oxalic acid solution+1% formalin until specimens are light blue, wash and fix with "hypo". Dehydrate with 95% and abs. alcohol and clear with oil of bergamot. Flatten the vessels and mount in balsam. Use dissecting microscope for the original dissection and flattening of the vessels.

Method B. Fresh tissue from animals. Perfuse the brain with physiological saline solution immediately after death till the blood is removed, then inject 500 cc. of the alcohol, acetic acid, formalin mixture (formula same as that used in method A). Remove the tissue and fix in the same solution 24 hr. Dissect out the blood vessels and wash and stain as in method A.—*H. A. Davenport.*

MOLLIER, G. **Eine Vierfachfärbung zur Darstellung glatter und quergestreifter Muskulatur und ihrer Beziehung zum Bindegewebe.** *Zts. wis. Mikr.*, **55**, 472–73. 1938.

This quadruple staining method gives excellent differentiation for the study of connective tissue muscle relationship. Practically any fixing fluid suitable for the "Azan" technic may be used for fixation.

(1) Transfer sections from 70% alcohol to Orcein (Tänzer-Unna) for 12 hr. (2) Rinse thoroly in dist. water until no color discharges. (3) Slightly overstain in Weigert's iron-haematoxylin, 1–3 min. (4) Rinse in dist. water. (5) Differentiate briefly in 1% HCl in 70% alcohol until nuclei are distinct, extracting excess orcein simultaneously. (6) Wash in tap water for 15 min. (7) Apply azocarmine G. 15–30 min., cold. Do not overstain. (8) Rinse in dist. water. (9) Transfer to 5% phosphotungstic acid, 3 changes for 2–6 hr. until collagen fibers are completely decolorized. (10) Rinse in dist. water. (11) Napthol green 15–30 min. (1 g. napthol green B (Hollborn) in 100 ml. dist. water plus 1 ml. glacial acetic acid). (12) Transfer directly to 95% alcohol (keeping sections in motion to prevent precipitation of the green) ½ min. (13) Run thru abs. alcohol, xylol, balsam. If elastic tissue stain is to be omitted begin at step 3.

Results: Chromatin and nucleoli stain pure deep blue; cytoplasm, bluish-red; myofibrils, deep red with sharp contours; elastic fibers, brown-black; mature collagen fibers, olive green; erythrocytes, brilliant red.—*J. M. Thuringer.*

RALSTON, MARTHA B., and WELLS, ARTHUR H. **Azocarmine stain for bone marrow.** *Am. Jour. Clin. Path., Tech. Suppl.*, **3**, 72–3. 1939.

Azocarmine, in combination with blue dyes, is most satisfactory for differential

staining of bone marrow. It gives vividly contrasting colors of myeloid elements and has the advantage that the cells are left in their *in vivo* relationships. The technic is as follows: Decalcify medullary bone over night in 5% acetic acid in Zenker's solution; wash 24 hr. in tap water; and prepare in paraffin by usual methods. Cut 4 μ sections, remove paraffin with xylene, and run thru alcohols to water. Heat azocarmine solution (Grubler's azocarmine, 1 g.; dist. water, 100 cc.: when dissolved add 1 cc. glacial acetic acid) in a paraffin bath at 56° C. Immerse slides 20 min. Wash in several changes of dist. water. Decolorize and differentiate in aniline alcohol (aniline, C.P., 2 drops; 95% alcohol, 50 cc.) followed by acid alcohol (95% alcohol, 100 cc., glacial acetic acid, 2 drops) until sections are rose red (30–90 sec.). Wash in dist. water. Stain 10–15 sec. in: 0.5% toluidine blue (C.I. 925), filtered just before use. Wipe off excess water. Dehydrate quickly in abs. alcohol. Modify slightly for different batches of stain.—*G. H. Chapman.*

SHERMAN, L., and SMITH, L. W. **Notes on the rubber paraffin method for embedding tissues.** *Amer. J. Clin. Path., Tech. Suppl.*, **2**, 171–3. 1938.

Peel sheets of pale crepe rubber (Quaker City Rubber Company, Philadelphia) into many layers and shred finely. To 160 g. of melted 53° or 58° C. paraffin at 78° C. add 20 g. (5 g. every 12 hr.) with frequent stirring until the colorless mixture is homogenous (about 4 days). Store at 55–60° C. Discard when the rubber separates out. For embedding, add 12.5 g. (varied according to desired consistency of block) to 500 g. of 53° or 58° C. paraffin and 5 g. of beeswax or bayberry wax at 60° C., stirring occasionally. It hardens and keeps well at room temp. (The stock mixture will not keep this way). Put back into the oven 24 hr. before use. The method is useful for making paraffin sections in hot weather, and particularly for eye sections and embryos. Standard dehydrating and clearing technics can be used.—*G. H. Chapman.*

PLANT MICROTECHNIC

GAUTHERET, R. **Sur la possibilité de realiser la culture indefinie de tissus de tubercules de carotte.** *Comp. Rend. Acad. Sci.*, **208**, 118–120. 1939.

Small portions of carrot tissue may be kept alive indefinitely in the following: Distilled water, 500 cc.; Knop solution, 500 cc.; mineral solution of Berthelot, or yeast ashes, 0.3 g.; glucose, 20.0 g.; gelatin, 13.0 g.; vitamin B_1, 0.001 g.; cysteine hydrochloride, 0.01 g.; indol B. acetic acid, 0.00001 g.

The cultures should be kept in diffuse light and free from necrotic areas and roots. Transplants should be made about every two months.—*E. Weier.*

KUWADA, Y., SHINKE, N., and OURA, G. **Artificial uncoiling of the chromonema spirals as a method of investigation of the chromosome structure.** *Zts. wiss. Mikr.*, **55**, 8–14. 1938.

By the use of a solution causing an alkaline reaction in plant spermatocytes, leaf epidermal nuclei, and pollen mother cells of *Tryxalis nasuta, Spiranthes australis, Impatiens Balsamina, Tradescantia reflexa,* etc., an attempt was made by the authors to dissolve the chromosome matrix and follow the steps in the process of uncoiling of chromonema spirals. Cells treated in this way previous to the usual acetocarmine stain show solid chromosome masses, irregularly drawn out spirals, and other stages of haplomitosis in much more detail than do ordinary preparations. The following chemicals were used: ammonia vapor, NH_4OH (1/100 mol), NH_4Cl (0.5 mol), NH_4SCN (0.5 mol), $(NH_4)_2SO_4$ (0.25–0.5 mol), KCN (2^{-7}, 2^{-8} mol), KOH (0.003 mol), NaOH (0.003 mol), NaCN (2^{-6}, 2^{-7} mol), Na_2HPO_4 (0.2 mol), NaOCl (2^{-6} mol), $NaHCO_3$ (2^{-8} mol).—*E. Barasch.*

LEITNER, J. **The use of acetocarmine in the study of the contents of mature, completely dried pollen grains.** *Zts. wiss. Mikr.*, **55**, 48–50. 1938.

Pollen grains are noted for retaining their germinative power a long time. The author shows the presence of one, two, or three cells within pollen grains as much as 112 years old. Due to the hardness of some of the husks, the grains could not be crushed and emptied. This was remedied by soaking them a few minutes in acetocarmine on a slide. The outlines of the sperm cells soon become recognizable. In 24 hr. they are as distinct as in fresh pollen grains. After breaking the husks, the staining time is no longer than with fresh grains. Species of the

families *Labiatas, Liliacea, Ranunculacea,* etc. were used for experiment.—*E. Barasch.*

LEFÈVRE, JOSEPH. **Similitude des actions cytologiques exercées par le phenylurethane et la colchicine sur des plantules végetales.** *Compt. Rend. Acad. Sci.*, **203**, 301–304. 1938.

Seeds of wheat, either before or during germination, were treated for 48 hr. at room temperature with vapors of phenylurethane, or with a saturated solution diluted 15 to 20 times. This treatment resulted in hypertrophy of the coleoptile, mesocotyl and root growing point, and finally in arrested growth. Mitosis was blocked and large nuclei with many chormosomes were formed.—*E. Weier.*

MICROÖRGANISMS

LIPP, J. **Detection of Spirochaeta pallida.** *Munch. Med. Woch.* **86**, 294. 1939.

A method is described for the detection of *Spirochaeta pallida* in primary lesions. Rub primary lesion with either swab and platinum loop or dry muslin until a clear serous exudate appears. Make a thin smear of the serum, dry in air (avoid heat or fixatives), stain 3–4 minutes with 3% aq. Victoria blue, wash, dry, and examine under oil immersion. The organisms appear sharply defined and stained a deep blue.—*L. Farber.*

RYO, EIHYO. **A simple method of staining bacterial flagella.** *Kitasato Arch. Exper. Med. (Tokyo).*, **14**, 218–220. 1937.

Smears from a young culture, dried and fixed as usual, are stained for 3–5 min. at room temp. in a combined mordant and stain prepared as follows. Soln. 1: 5% phenol, 10 cc.; powdered tannin, 2 g.; sat. soln. potas. alum, 10 cc. Soln. 2: sat. alc. soln. of crystal violet. For use, 10 parts of Soln. 1 and 1 part of Soln. 2 are thoroly mixed at the moment of staining. After staining the solution is washed off, and the preparation mounted in cedar oil. The solutions, before mixing, can be preserved a long time.—*H. J. Conn.*

T'UNG, T. **Photodynamic action of safranine on Gram-negative bacilli.** *Proc. Soc. Exp. Biol. & Med.*, **39**, 415–7. 1938.

Safranin exhibited a photodynamic action on Gram-negative organisms. Previous experiments with other dyes failed with this group of organisms. Sat. aq. safranin O (water-soluble, National Aniline and Chemical Co.) was diluted in water adjusted to pH 8.0 with NaOH. Organisms (22 strains of various species) were mixed with dye and exposed to light for 2 hr. The maximum action was obtained with 1% safranin. Both weaker and stronger concentrations were less effective. Unexposed cultures were in most cases uninjured by safranin alone. The photodynamic action as such was increased in experiments in which 0.15% H_2O_2 was used. It is suggested that oxidation may play a rôle in photodynamic action.—*M. S. Marshall.*

HISTOCHEMISTRY

CARERE-COMES, O. **New methods for the histochemical demonstration of potassium, and selective staining of tissues rich in potassium.** *Zts. wiss. Mikr.*, **55**, 1–6. 1938.

The author finds that sodium paradipicrylamine sold as "siena orange" by Dr. K. Hollborn & Sons, Leipzig S3, ready for use, stains tissues selectively and quantitatively for potassium, by the following technic (1) Treat sections that have been fixed in neutral formol solution and embedded in paraffin as follows: remove paraffin, place in 100%, 90%, 70% alcohol, dist. water, (2) Siena orange solution, 2 min.; 10% HCl, 3 min.; wash in twice renewed dist. water, 10 min.; dry at 37°C. or with filter paper and cover with thickened cedar oil. The tissues containing potassium stain orange; others stain pale yellow, or not at all.

Contrasting stain for muscle fibers: repeat (1) above, place in siena soln., 2 min.; 10% HCl, 3 min.; aq. acetone, 5–15 min.; aniline blue for contrast (0.01% aq. soln. to be freshly prepared from 1% stock soln.), 10–15 min. Wash in dist. water, dry with filter paper, clear with abs. alcohol (quickly) or oil of cloves and xylol, and embed in neutral Canada balsam (Hollborn) or thick cedar oil. The orange muscle fibers show up well.

Contrasting stain for hemopoietic organs: (1) as above; place in siena orange solution, 1 min.; 10% HCl, 3 min.; dist. water, twice renewed, 10 min.; differentiate carefully with dil. acetone, 5–10 min.; stain with gentian violet (10 drops of sat. alc. soln. in 20 cc. dist. water), 2 min. Wash with dist. water and dry with filter paper. Differentiate carefully in abs. alcohol. Clear with xylol, embed in neutral Canada balsam. Erythrocytes appear orange.—*E. Barasch.*

KELLEY, E. G. **Reactions of dyes with cell substances. V. Differential basic combination of tissue nuclei with special reference to resting and mitotic cells of tumor tissue.** *J. Biol. Chem.*, **127**, 73–86. 1939.

This method has been developed in an attempt to indicate the amount of nucleic acid in the nucleus. Nuclei with greater nucleic acid content bind more basic dye at the same pH. The complete staining of the nucleus is the point of measurement recorded. For example, the nuclei of thymocytes have less nucleic acid than the nuclei of lymphocytes; nuclei in mitosis more than resting nuclei. Acid and basic dyes were made up at a concentration of 0.1% of actual dye by dissolving weighed amounts of the dye in 50 cc. portions of buffer solutions. Thionine was used in Kolthoff's borax-succinic acid buffers from pH 3.0–5.8, also in dilute HCl and acetate buffers to cover range from pH 2.0–6.0. Toluidine blue was soluble in all the buffers, but was used mostly in Sörensen's citrate HCl and NaOH buffers to cover the range from pH 2.0–7.0. Fast green was soluble in all buffers. All dyes were from certified batches. Buffers were made up at 0.2 pH-intervals and their pH-values determined to ± 0.02 pH by means of a vacuum tube glass electrode. Likewise the dye solutions were tested to ± 0.02 pH. Tissues were fixed in: alcohol (strength not given); alcohol containing 5% acetic acid; mercuric chloride at pH 2.0 and 6.0; phosphotungstic acid at pH 2.0–6.0. Sections from material were embedded in paraffin, cut at 7μ, and passed from xylol thru alcohols to distilled water. Individual slides were transferred for 15 min. to a 35×80 mm. glass shell vial containing 50 cc. of a buffer solution of same pH as acid and basic dye solutions . They were then rinsed in distilled water; transferred to 0.1% dye-buffer solution for 1 minute; rinsed in distilled water; transferred to 0.1% acid dye-buffer solution for 1 minute; rinsed in distilled water; dipped in 95% alcohol until no further running of dye could be observed; placed in 100% alcohol; and mounted in balsam after passing thru xylol. Sections were compared with a comparision microscope. A Wratten filter (B2) is recommended for better comparison.—*J. E. Kindred.*

KELLEY, E. G. **Reaction of dyes with cell substances. IV. Quantitative comparison of tissue nuclei and extracted nucleoproteins.** *J. Biol. Chem.*, **127**, 55–71. 1939.

Extraction of nucleoproteins from three different types of rat tissue (sarcoma, carcinoma, and thymus gland) shows that substances of constant composition can be obtained from each tissue. Each tissue yields materials with the same ratio of nucleic acid to protein, and the same isoelectric point. Toluidine blue in aqueous solutions of 0.1% and 0.005% is also used as a means of testing the similarity of tissue nuclei and extracted nucleoproteins. These tests show a complete lack of correlation between the isoelectric point of tissue and that of the extracted protein. These experiments show, however, that in the case of alcohol-fixed tissues the amount of dye taken up follows very closely the relative nucleic acid content of the nucleoproteins in the nucleus. This cannot be said about $HgCl_2$ or formalin fixation.—*J. A. de Tomasi.*

LANCASTER, SARAH. **Nature of chromaffin nerve cells in certain annulates and arthropods.** *Trans. Amer. Micr. Soc.*, **58**, 90–6. 1939.

Neither methylene blue, used as a vital stain, nor $FeCl_3$, NaOH, $CuSO_4$, or KCN gave indication, by precipitation tests, of an adrenalin-like substance in living giant nerve cells of earthworms, grasshoppers, or leeches. In all cases the cords were fixed in Orth's fluid, stained with Heidenhain's Fe alum hematoxylin, and Gage's acid fuchsin as a counterstain. In the same organisms, however, giant chromaffin nerve cells were found that gave positive reactions to the chromate tests for adrenalin. In fixed tissues, the chromaffin reaction made the cytoplasm appear denser than that in the surrounding regions. Findings support the assumption that these giant chromaffin nerve cells possess dual properties of nervous and adrenal cells, and may represent the origin of both the sympathetic nervous and the adrenal systems in vertebrates.—*J. A. de Tomasi.*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stains approved since the last one listed in the April number of this Journal.

STAINS CERTIFIED MAR. 1, 1939 TO MAY 31, 1939*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Orange G	CO–6	78%	As counterstain in histology	Mar. 3, 1939
Eosin Y	DE–3	81%	For use in bacteriological media	Mar. 18, 1939
Basic fuchsin	DF–5	93%	For use in bacteriological media	Mar. 29, 1939
Methyl violet 2B	NMv–6	87%	As histological and bacteriological stain	Mar. 30, 1939
Brilliant green	NBg–8	89%	For use in bacteriological media	Apr. 25, 1939
Giemsa stain	GGe–6	....	As blood stain	May 10, 1939
Giemsa stain	NGe–5	....	As blood stain	May 22, 1939
Cresyl violet	NW–7	93%	For use in histology	May 22, 1939
Neutral red	NX–7	65%	As histological and vital blood stain	May 25, 1939

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.